Advanced 3D human tissue models and testing services for cosmetic, consumer product and pharmaceutical industry

The ATERA – RHE skin irritation test method

Poster N.166

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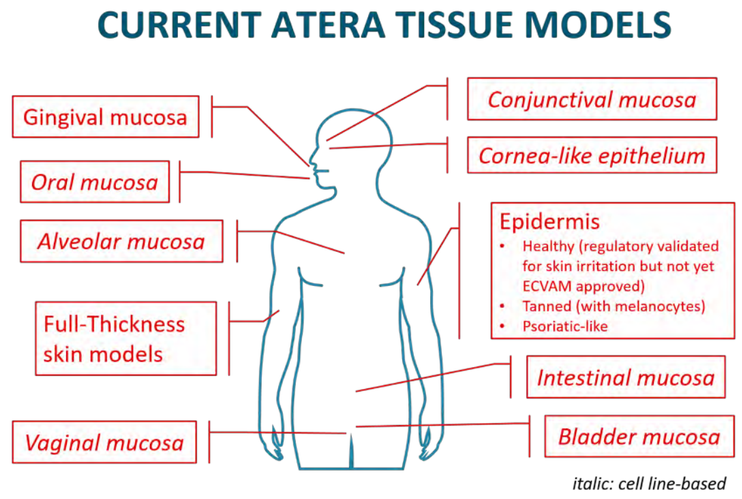
INTRODUCTION

In line with industrial innovation and many EU regulatory encouragements or requirements (EC 1223/2009 Regulation on cosmetic products, 2010/63/EU Directive on the protection of animals, EC 1272/2008 Regulation CLP , EC 1907/2006 Regulation REACH, 98/8/EC Regulation Biocidal products), the need for reconstructed human tissue models is still increasing. Advanced Tissue Engineering for Research Applications (ATERA) is a new tissue engineering company specialized in the development, validation, manufacturing and commercialization of "advanced" human tissue models. These models can be used as a reliable, cost-effective, and predictive alternative to animal experimentation in product evaluation, industrial safety and efficacy testing as well as medical research.

MATERIALS & METHODS

All human tissue models are produced in a ISO 9001/V2008 certified state-of-the-art production facility (Sterlab, Vallauris, France).

To show the capacity of the **ATERA – Reconstructed Human Epidermis (RHE)** skin irritation test method to replace the *in vivo* Draize test for regulatory accepted testing, a catch up validation study was performed, according to the performance standards (PS) for the Skin Irritation Test Methods using RHE as described in the OECD test guideline 439, and comprised of testing 20 reference chemicals in three independent laboratories (Sterlab (F), VITO (B), VitroScreen (I)) under blinded conditions [1, 2].



Vascularized skin equivalent: Briefly, a biological vascularized scaffold (BioVaSc) was seeded with human fibroblasts, keratinocytes, and human microvascular endothelial cells and cultured for 14 days at the air-liquid interface. The formation of the skin barrier was measured non-destructively using impedance spectroscopy.

RESULTS

Performance Standards (PS) validation study of ATERA's RhE method for skin irritation testing as described in TG 439:

A test substance is considered to be irritant to skin (Cat. 2) if the mean relative viability after 42 minutes exposure and 42 hours post incubation is less or equal to 50% of the negative control (Prediction model: Table 1). The acceptance criteria for both the negative control (NC; PBS) and the positive control (PC; SDS 5%) were met: the mean OD of the NC was ≥ 0.8. The mean viability of the PC was well below the acceptance threshold of 40% (range: 1.0% to 11.2%). The SD of the PC's was well below the acceptance limit of 18% (Table 2). For the 20 test compounds, all acceptance criteria were met showing the high predictive capacity and reliability of the ATERA – RHE test method that archived an overall accuracy of 85% and reproducibility of 93% (Table 3 and Table 4).

SkinVasc: Testing services are also performed on tissue models that are currently not mass produced. The vascularized skin equivalent, SkinVasc developed at Fraunhofer IGB [3], revealed a specific histological architecture representative of the human dermis and epidermis (Fig 2 A,B). Endothelial cells lined the walls of the formed vessels (Fig 2C) that could be perfused with a physiological volume flow. The epidermal barrier formation was assessed by impedance spectroscopy (Fig 3A). During culture, the surface-normalized ohmic resistance increased up to 832 Ω*cm² and the normalized capacitance decreased to 1.43 µF/cm² (Fig 3B). SkinVasc model allows to evaluate both systemic efficacy and toxicity of test compounds, in the presence or absence of circulation of other cell types (e.g. different immune cells) thus providing a new in vitro tool helpful to develop candidate methods assessing potential systemic toxicity.

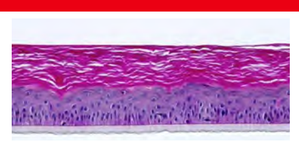


Figure 1. H&E image of the ATERA-RHE model

In vitro Result	Classification (*In vivo* Prediction)
Mean tissue viability >50%	No Category (No Cat.)
Mean tissue viability ≤50%	Category 2 (Cat. 2)

Table 1. Summary statistics for the controls

Laboratory	Run	NC (OD) Mean	PC (Viability, %) Mean	PC (Viability, %) SD
Sterlab	1	1.0 [A]	3.8	0.3
	2	1.1	11.2	1.8
	3	1.0	7.6	4.7
	4	1.1	5.3	2.7
VITO	1	1.1	1.5	0.2
	2	1.3	1.0	0.1
	3	1.4	1.9	0.4
	4	1.2	1.6	0.1
Vitroscreen	1	0.8	2.3	0.0
	2	0.9	2.4	0.2
	3	1.0	2.1	0.6

[A] mean of two tissues

Table 2. Summary statistics for the controls

No.	UN GHS	Chemical	Sterlab Viability (%)	Sterlab Prediction	VITO Viability (%)	VITO Prediction	VITROSCREEN Viability (%)	VITROSCREEN Prediction	Concordance
1	No Cat.	1-bromo-4-chlorobutane	15.4	Cat. 2	28.1	Cat. 2	6.5	Cat. 2	Yes
2	No Cat.	4-methyl-thio-benzaldehyde	38.7	Cat. 2	3.7	Cat. 2	2.7	Cat. 2	Yes
3	No Cat.	allyl phenoxy-acetate	102.9	No Cat.	85.0	No Cat.	53.3	No Cat.	Yes
4	No Cat.	cinnamaldehyde	0.1	Cat. 2	0.7	Cat. 2	1.1	Cat. 2	Yes
5	No Cat.	diethyl phthalate	87.3	No Cat.	100.0	No Cat.	83.8	No Cat.	Yes
6	No Cat.	heptyl butyrate	77.1	No Cat.	74.3	No Cat.	82.4	No Cat.	Yes
7	No Cat.	hexyl salicylate	95.9	No Cat.	86.7	No Cat.	84.6	No Cat.	Yes
8	No Cat.	isopropanol	101.1	No Cat.	93.7	No Cat.	77.4	No Cat.	Yes
9	No Cat.	methyl stearate	101.2	No Cat.	105.5	No Cat.	98.5	No Cat.	Yes
10	No Cat.	naphthalene acetic acid	109.3	No Cat.	96.6	No Cat.	78.0	No Cat.	Yes
11	Cat. 2	1-bromohexane	9.1	Cat. 2	5.7	Cat. 2	2.1	Cat. 2	Yes
12	Cat. 2	1-decanol	3.8	Cat. 2	3.9	Cat. 2	2.2	Cat. 2	Yes
13	Cat. 2	1-methyl-3-phenyl-1 piperazine	2.1	Cat. 2	10.2	Cat. 2	1.4	Cat. 2	Yes
14	Cat. 2	2-chloromethyl-3,5-dimethyl4-methoxypyridine HCl	2.0	Cat. 2	1.0	Cat. 2	1.1	Cat. 2	Yes
15	Cat. 2	benzenethiol, 5-(1,1dimethylethyl)-2-methyl	9.7	Cat. 2	5.4	Cat. 2	11.2	Cat. 2	Yes
16	Cat. 2	cyclamen aldehyde	1.8	Cat. 2	9.5	Cat. 2	1.2	Cat. 2	Yes
17	Cat. 2	di-n-propyl disulphide	11.6	Cat. 2	8.8	Cat. 2	2.1	Cat. 2	Yes
18	Cat. 2	heptanal	16.6	Cat. 2	9.4	Cat. 2	1.8	Cat. 2	Yes
19	Cat. 2	potassium hydroxide (5% aq.)	13.0	Cat. 2	0.5	Cat. 2	1.9	Cat. 2	Yes
20	Cat. 2	tetrachloroethylene	2.8	Cat. 2	1.6	Cat. 2	1.3	Cat. 2	Yes

Viability corresponds with the mean of three independent runs

Table 3. Between laboratory reproducibility: concordance in predictions

Reference	Overall Cat. 2	Overall No Cat.	Overall Total	Sterlab Cat. 2	Sterlab No Cat.	Sterlab Total	VITO Cat. 2	VITO No Cat.	VITO Total	Vitroscreen Cat. 2	Vitroscreen No Cat.	Vitroscreen Total
Cat.2	**30**	0	30	**10**	0	10	**10**	0	10	**10**	0	10
No Cat.	9	**21**	30	3	**7**	10	3	**7**	10	3	**7**	10
Total	39	21	60	13	7	20	13	7	20	13	7	20
Sensitivity (%)		100			100			100			100	
FNR (%)		0			0			0			0	
Specificity (%)		70			70			70			70	
FPR (%)		30			30			30			30	
Accuracy (%)		85			85			85			85	

Table 4. Performance of ATERA-RHE test method

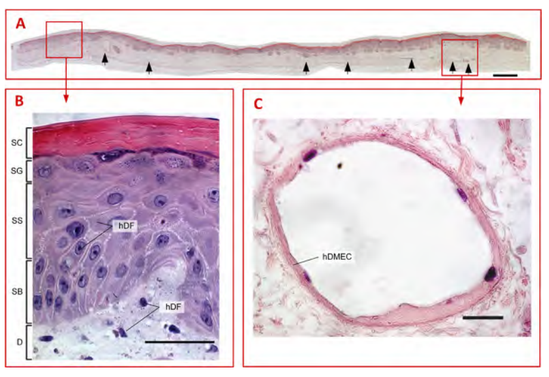


Figure 2. H&E images of the ATERA-SkinVasc model

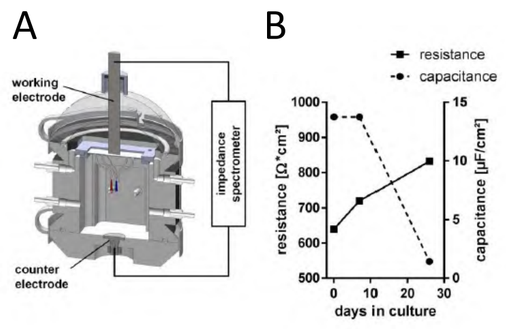


Figure 3. Functional characterization of the vascularized skin equivalent

CONCLUSION

Advanced Tissue Engineering for Research Applications provides a set of unique tissue models allowing the development of advanced alternative methods to animal testing including novel predictive tools that are more relevant to human health evaluation.

REFERENCES

[1]- OECD (2015), Performance Standards for the Assessment of Proposed Similar or Modified In Vitro Reconstructed Human Epidermis (RhE) Test Methods for Skin Irritation Testing as described in TG439. Series on Testing and Assessment No. 220, OECD, Paris.

[2]-OECD (2015), OECD Guideline for the Testing of Chemicals No. 439. In vitro skin irritation: reconstructed human epidermis test method. OECD, Paris.

[3]- ALTEX. 2016 May 15. A first vascularized skin equivalent as an alternative to animal experimentation. Groeber F. et al. doi: 10.14573/altex.1604041

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